

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor(s): Karla Robotti

Serial No.: 10/072,525

Examiner: Quang Nguyen

Filing Date: February 5, 2002

Group Art Unit: 1633

Title: METHOD OF IMMOBILIZING BIOLOGICALLY ACTIVE MOLECULES FOR ASSAY PURPOSES IN A MICROFLUIDIC FORMAT

COMMISSIONER FOR PATENTS
P.O. Box 1450
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TRANSMITTAL OF APPEAL BRIEF

Sir:

Transmitted herewith is the Appeal Brief in this application with respect to the Notice of Appeal filed on July 16, 2008.

The fee for filing this Appeal Brief is (37 CFR 1.17(c)) **\$510.00**.

(complete (a) or (b) as applicable)

The proceedings herein are for a patent application and the provisions of 37 CFR 1.136(a) apply.

☐ (a) Applicant petitions for an extension of time under 37 CFR 1.136 (fees: 37 CFR 1.17(a)(1)-(5)) for the total number of months checked below:

<input type="checkbox"/>	one month	\$ 120.00
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☐ The extension fee has already been filled in this application.

☒ (b) Applicant believes that no extension of term is required. However, this conditional petition is being made to provide for the possibility that applicant has inadvertently overlooked the need for a petition and fee for extension of time.

Please charge to Deposit Account **50-1078** the sum of **\$510.00**. At any time during the pendency of this application, please charge any fees required or credit any overpayment to Deposit Account **50-1078** pursuant to 37 CFR 1.25.

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Respectfully submitted,

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Date: September 16, 2008

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**IN THE UNITED STATES
PATENT AND TRADEMARK OFFICE**

Appl. No. : 10/072,525
Applicant(s): Karla Robotti
Filed: February 5, 2002
TC/A.U.: 1600/1633
Examiner: Quang Nguyen
Atty. Docket: 10011206-01
Confirmation No.: 2898
Title: Method of immobilizing Biologically Active
Molecules for Assay Purposes in a
Microfluidic Format

APPEAL BRIEF

Honorable Assistant Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

In connection with the Notice of Appeal dated July 16, 2008, Applicant provides the following Appeal Brief in the above-captioned application.

TABLE OF CASES

1. *KSR Int'l Co. v. Teleflex Inc.*, 127 S. Ct. 1727; 82 U.S.P.Q.2D 1385 (2007)
2. *Graham v. John Deere Co.*, 383 U.S. 1, 17, 148 USPQ 459, 467 (1966)
3. *Monroe Auto Equipment Co. v. Heckethorn Mfg. & Supply Co.*, 332 F.2d 406,
4. *Ex parte Crawford, et al.* Appeal 20062429, May 30, 2007

1. Real Party in Interest

The real party in interest as assignee of the entire right and title to the invention described in the present application is Agilent Technologies, Inc., having a principle place of business at 5301 Stevens Creek Blvd, Santa Clara, CA USA.

2. Related Appeals and Interferences

There are no known related appeals or interferences at this time.

3. Status of the Claims

Claim 1-3, 9, 15-21,24,26-42,44-56,58 and 59 are pending in this application. Claims 4-8, 10-14, 22, 23, 25,43 and 57 are cancelled. Claims 1-3,9, 15-21,24,26-42,44-56,58 and 59 are the subject of the present Appeal. Claims 1-3, 9, 15-21,24,26-42,44-56,58 and 59 are finally rejected, and are duplicated in the Appendix.

4. Status of the Amendments

A final Office Action on the merits was mailed on March 18, 2008. A Reply under Rule 116 was filed on May 19, 2008. An Advisory Action was mailed on June 16, 2008. There are no pending amendments with respect to this application.

5. Summary of the Claimed Subject Matter¹

Referring to Claim 44:

In accordance with an embodiment, a method of preparing a microanalytical device (See, for example page 7, lines 11-19; and ‘V. Microfluidic Systems’ beginning on page 22) comprises forming a sol-gel (See for example ‘II-Sol-Gel Technology’ beginning on page 11) comprising an entrapped biological molecule (See, for example

¹ In the description to follow, citations to various reference numerals, drawings, and corresponding text in the specification are provided solely to comply with Patent Office rules. It is emphasized that these reference numerals, drawings, and text are representative in nature, and not in any way limiting of the true scope of the claims. It would therefore be improper to import anything into any of the claims simply on the basis of illustrative language that is provided here only under the obligation to satisfy Patent Office rules for maintaining an Appeal.

Fig. 1; page 7, lines 11-19; 'III. Sol-Gel Immobilization of Biomolecules;' IV. Biomolecules' beginning on page 16; and the Example beginning at the bottom of page 30 of the filed application). The method comprises crushing the sol-gel to particulates having a diameter of from about 10 μm to about 80 μm and forming the sol-gel particulates into a bed within the microanalytical device or on the surface of the microanalytical device (See, for example 'VI. Methods of Use' beginning on page 26.).

Referring to claim 58:

In accordance with a representative embodiment, in a microanalytical device comprised of a substrate (See, for example 'V. Microfluidic Systems' beginning on page 22) and at least one feature selected from microchannels, microcolumns, and combinations thereof (See, for example page 14, line 28 through page 15, line 9). The improvement comprises incorporating into said at least one feature (e.g., microchannels, microchannels or combinations thereof) and/or onto a surface of the substrate (e.g., as described in 'V. Microfluidic Systems) a sol-gel (See for example 'II-Sol-Gel Technology' beginning on page 11) having a biological molecule entrapped therein (See, for example Fig. 1; page 7, lines 11-19; 'III. Sol-Gel Immobilization of Biomolecules;' IV. Biomolecules' beginning on page 16; and the Example beginning at the bottom of page 30 of the filed application). The sol-gel has been crushed into particulates having a diameter of from about 10 μm to about 80 μm and the particulates have been formed into a bed within the microanalytical device or on the surface of the microanalytical device (See, for example 'VI. Methods of Use' beginning on page 26.).

6. Grounds of Rejection to be Reviewed on Appeal

The issues in the present matter are whether:

I. Claims 1-3,24,26,27,33-36,41-42,44-55 and 58-59 are properly rejected under 35 U.S.C. § 103(a) as being unpatentable over in view of *Dunn, et al.* (U.S. Patent 5,200,334); *Lochhead, et al.* (U.S. Patent 6,039,897); *Anvir, et al.* (U.S. Patent 5,300,564); *Anvir, et al.* (U.S. Patent 6,159,453); and *Swedeberg, et al.* (6,240,790); and

II. Claims 1,9, 15-21, 28-32,37-40 and 44 are rejected under 35 U.S.C. § 103(a) as being obvious in view of *Dunn, et al.* (U.S. Patent 5,200,334); *Lochhead, et al.* (U.S. Patent 6,039,897); *Anvir, et al.* (U.S. Patent 5,300,564); *Anvir, et al.* (U.S. Patent 6,159,453); *Swedeberg, et al.* (6,240,790); and *Reetz, et al.* (Biotechnology and Bioengineering, Vol. 9:527-534, 1996).

7. Argument

In this portion of the Appeal Brief, arguments are provided. Notably, wherever applicable, Applicant maintains previous arguments for patentability provided in responses to Office Actions.

a. Rejections under 35 U.S.C. § 103(a)

As stated in MPEP § 2143, in order to establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations.

In *KSR Int'l Co. v. Teleflex Inc.*, 127 S. Ct. 1727; 82 U.S.P.Q.2D 1385 (2007), the Court stated “A factfinder should be aware, of course, of the distortion caused by hindsight bias and must be cautious of arguments reliant upon *ex post* reasoning. See *Graham v. John Deere Co.*, 383 U.S. 1, 17, 148 USPQ 459, 467 (1966) (warning against a “temptation to read into the prior art the teachings of the invention in issue” and instructing courts to “guard against slipping into the use of hindsight” (quoting *Monroe Auto Equipment Co. v. Heckethorn Mfg. & Supply Co.*, 332 F.2d 406, 412 (CA6 1964))).” Moreover, if there is no suggestion to combine the teachings of the applied art, other than the use of Applicants’ invention as a template for its own reconstruction, a rejection for obviousness is improper. *Ex parte Crawford, et al.* Appeal 20062429, May 30, 2007. In furtherance to the need for the suggestion to combine the teachings of the applied art, it is established that rejections on obviousness grounds cannot be sustained by

mere conclusory statements: instead there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness. *KSR Int'l v. Teleflex*, 127 S. Ct. at 1741.

I. Rejection of claims 1-3,24,26,27,33-36,41-44, 46-55 and 58-59 under 35 U.S.C. § 103(a)

Claim 44 recites:

A method of preparing a microanalytical device, comprising forming a sol-gel comprising an entrapped biological molecule, crushing the sol-gel to particulates having a diameter of from about 10 μm to about 80 μm , and forming the sol-gel particulates into a bed within the microanalytical device or on the surface of the microanalytical device.

Claim 58 recites:

In a microanalytical device comprised of a substrate and at least one feature selected from microchannels, microcolumns, and combinations thereof, the improvement which comprises incorporating into said at least one feature and/or onto a surface of the substrate a sol-gel having a biological molecule entrapped therein, wherein the sol-gel has been crushed into particulates having a diameter of from about 10 μm to about 80 μm and the particulates have been formed into a bed within the microanalytical device or on the surface of the microanalytical device.

i. Dunn, et al. and Lochhead, et al.

The Office Action initially turns to *Dunn, et al.* for its disclosure of its assertion of the advantageous aspects of encapsulating enzymes in a porous, transparent glass structure, such as, such structures prepared by the sol-gel process. Such an encapsulation would be significantly easier to miniaturize and would be far less cumbersome and far more reliable than membrane encapsulation systems. Thus, while the reference describes forward-looking benefits of encapsulating enzymes in porous transparent glass structures (e.g., by sol-gel), there is no suggestion of *a sol-gel comprising an entrapped biological*

molecule or of crushing the sol-gel to particulates as featured in claim 44; or a biological molecule entrapped in sol-gel and the sol-gel has been crushed into particulates as claimed in claim 58.

The Office Action concedes that the primary reference to *Dunn, et al.* fails to disclose certain features of the claims. Specifically:

“Dunn et al. does not teach explicitly a method of preparing any microanalytical device containing sol-gel particulates comprising an entrapped biological molecule and having a diameter of from about 10 micrometers to about 80 micrometers, or a method of using the same microanalytical device.”
(Emphasis in original.)

The Office Action then directs Applicant to *Lochhead, et al.* for the alleged disclosures of the features not disclosed in *Dunn, et al.* The Office Action states:

“...Lochhead et al already disclosed a Micro-molding in capillaries (MIMIC) process for fabricating micronscale structures or devices for use in sensor, waveguide and integrated optics applications using a micro-molding fluid that is a sol that can comprise a variety of biologically active molecules including proteins, enzymes, antibodies, antigens and nucleic acid which bind to, or interact with analytes including other biologically active molecules (see at least col. 6, lines 9-62). Lochhead et al further taught an exemplified fluid channel that is an element of a micro-fluidic chemical analysis system with appropriate means for fluid sample introduction and a means for detecting indicator response to a particular analyte that may be present in fluid passed through the micro channel (see Fig. 5, and col. 9, lines 37-62).” (Emphasis in original.)

The reference to *Lochhead, et al.* relates to integrated optics applications using micro-molding in capillaries (MMIC) by casting patterns from micro-molding fluids that are sols. While the reference mentions the interest in microchip based technologies for

analysis of biological fluids, *Lochhead, et al.* fails to cure the deficiencies of *Dunn, et al.* Claims 44 and 58 feature *a sol-gel having a biological molecule entrapped in the sol-gel* and incorporated into *microchannels, microcolumns, and combinations thereof*. Thus, casting patterns from micro-molding fluids that are sols is described in *Lochhead, et al.*, but the sol-gels of the present claims are not related to the microchannels or microcolumns of a microanalytic device, but rather as an entrapment material for biological molecules. Thus, the reference to *Lochhead, et al.* seems to be merely cumulative to that of *Dunn, et al.* in the sense that both see advantages in encapsulating enzymes and the like in a sol-gel glass. But, neither disclose the sol-gel particulates as specifically recited in claims 44 and 58.

In support of their position, Applicants direct attention to column 5, lines 39-49, which describes Fig. 1E:

“FIG. 1E illustrates the single-step MIMIC method. The master of FIG. 1D (20) is positioned channel side-down on the substrate (25) and micro-molding fluid (the precursor to surface patterned material) is introduced into independent reservoirs of the elastomeric master. Fluids, including sols, fill the channel that extends from the reservoir by capillary action. Because the channels are not interconnected to each other and because of the separate reservoirs present in the mold, each channel can be filled with a different micro-molding fluid. The filled mold is aged, heated, irradiated or otherwise processed to form desired solid materials in the channels adhered to the substrate. “

Moreover, while the reference does disclose biologically active materials in the micro-molding fluids, this again differs from features of claims 44 and 58. Specifically, the sol-gels of claims 44 and 58 comprise *biological molecule entrapped* in the sol-gel particulates, whereas the micro-molding fluids with sol-gel entrapping cells are used to form the MIMIC capillaries. In particular, as described at column 6, lines 41-49 of *Lochhead, et al.*:

Micro-molding fluids can comprise a variety of biologically active molecules including proteins, enzymes, antibodies, antigens and nucleic acid which bind to, or otherwise react or interact with analytes including other biologically active molecules.

Micro-molding fluids can further comprise whole biological cells and/or cell fractions (e.g., cell wall preparations and the like.) Whole cells have been successfully entrapped in sol-gel systems.

Thus, the reference discloses the use of sol-gels to fabricate MIMIC by casting patterns from micro-molding fluids that are sols, but lacks at least the disclosure at least of *preparing a microanalytical device, comprising...forming the sol-gel particulates (comprising an entrapped biological molecule) into a bed within the microanalytical device or on the surface of the microanalytical device; or*

microchannels, microcolumns, and combinations thereof, incorporating into the microchannels or microcolumns and/or onto a surface of the substrate of the microanalytic device a sol-gel having a biological molecule entrapped therein.

Moreover, the Office Action fails to provide an articulated rationale to support a legal conclusion of obviousness. Specifically, even assuming arguendo that the teachings of *Lochhead, et al.* cured to admitted deficiencies of *Dunn, et al.*, there is no clearly articulated motivation to transplant the sol-gel used as the micro-molding fluid from *Lochhead, et al.* to the sol-gel particulates comprising entrapped biological molecules into a bed within the microanalytical device or surface thereof; or into the microchannels or microcolumns and/or onto a surface of the substrate microanalytic device. Rather, at page 8, the (final) the Office Action merely states:

Lochead et al. already taught the feasibility of **fabricating micron-scale devices containing biological material embedded in a sol-gel at least for sensor, waveguide and integrated optic applications, and microfabricated devices for applications ranging from remote chemical sensing to medical analysis due to the potential of rapid analysis and portability**...In summary, an ordinarily skilled artisan would have been motivated to carry out the above modification because the reduction in size of an analytical procedure of technique translates to a reduction in analysis time, costs and paves the way for high throughput applications. (Emphasis in original).

Thus, there is no clearly articulate motivation for the transplantation of the sol-gel used as the micro-molding fluid from *Lochhead, et al.* to realize the sol-gel particulates

comprising entrapped biological molecules into a bed within the microanalytical device or surface thereof; or into the microchannels or microcolumns and/or onto a surface of the substrate microanalytic device. Nor could there be: the neither reference teaches the claimed particulates. As a result, the rejection on obviousness grounds is alleged based on mere conclusory statements and not the requisite articulated reasoning with some rational underpinning (i.e., basis to transplant the teachings of *Lochhead, et al.*) to support the legal conclusion of obviousness.

ii. Anvir references

The Office Action then directs attention to *Anvir, et al.* ('564) and *Anvir, et al.* ('453). While the Office Action does not recite with clarity and specificity how the first two references in the rejection are deficient, the '564 reference is relied upon for the disclosure of sol-gel entrapped enzymes. Notably, at page 6, the Office Action states:

Particularly, Anvir, et al. taught that for sol-gel immobilized enzymes, crushed powder sol gel glasses may be used to support enzymatic column chromatography. (col. 5, lines 37-39 and col. 7, lines 55-57). (Emphasis in original).

At the outset, Applicants note that proteins are entrapped in a matrix of a forming sol-gel (see column 5, lines 21-22); and thus there is no clear disclosure of the biological molecules entrapped in sol-gel particulates as claimed. True, the reference does describe ground sol-gel glass in column 7 as relied upon in the Office Action. However, the reference explicitly states at lines 62-63 "It was found that neither significant enzyme activity or nor protein could be detected in the eluates." While other entrapments are described (e.g., trypsin), the *sol-gel having a biological molecule entrapped therein*, and *the sol-gel having been crushed into particulates* is not disclosed in the '564 reference.

The Office Action directs Applicants to *Anvir, et al.* ('453) for the alleged disclosure of the claimed particulates of specified diameter. Applicants have noted that the '453 reference is directed to doped sun-screen agents. The reference discloses sol-gel matrices may be particles having a diameter of 0.01 μm to 100 μm . There is no

disclosure of biological molecules entrapped therein, or the disclosure or suggestion for use of the sol-gel for anything other than sunscreen. The latter omission is particularly interesting considering that at least one inventor is common (Anvir) to both the '453 and the '564 patents. Most notably, if anyone would have had the foresight to extend the use of the sol-gel particulates of US Patent 6,159,453 to *microanalytical devices* and their preparation, it serves to reason that the common inventor, David Anvir, would have, given his endeavors related to US Patent 5,300,564. Yet, there is no disclosure, teaching or suggestion found within the four corners of the '453 patent of such applications. Thus, Applicants respectfully submit that not only does the application of the teachings of *Anvir, et al.* '453 to the claims under present examination require impermissible hindsight, but also, there cannot possibly be a motivation to combine *Anvir, et al.* '453 that does not rely on such hindsight and Applicants' claims as templates for their own reconstruction. Furthermore, because the inventor who worked in both fields of endeavor as described in the '564 and '453 patents did not transplant the concepts of the '453 patent to microanalytical devices and their preparation, the rejection on obviousness grounds based on *Anvir, et al.* '453 cannot include the requisite articulated reasoning with some rational underpinning (i.e., basis to transplant the teachings of the '453 reference into those of the '564 reference) to support the legal conclusion of obviousness.

b. Rejections Improper

For at least the reasons set forth above, Applicant respectfully submits that the applied art fails to disclose at least one feature of claims 44 and 58, and the combination of references is improper. Therefore, a proper *prima facie* case of obviousness has not been established. Therefore, claims 44 and 58 are patentable over the applied art. Moreover, claims 1-3, 24, 26, 27,33-36,41-43, 46-55 and 59, which depend variously from claims 44 and 58, are patentable for at least the same reasons.

II. Rejection of claims 1,9, 15-21, 28-32,37-40 and 44 under 35 U.S.C. § 103(a)

The rejection of claim 44 is based on the applied art to *Dunn, et al.* (U.S. Patent 5,200,334); *Lochhead, et al.* (U.S. Patent 6,039,897); *Anvir, et al.* (U.S. Patent

5,300,564); *Anvir, et al.* (U.S. Patent 6,159,453); *Swedeberg, et al.* (6,240,790) in the same manner as I. above. Therefore, in the interest of brevity, Applicants refer the Board to the traversal of this rejection set forth in detail in I. above.

For at least the reasons set forth above, Applicant respectfully submits that the applied art fails to disclose at least one feature of claim 44, and the combination of references is improper. Therefore, a proper *prima facie* case of obviousness has not been established. Therefore, claim 44 is patentable over the applied art. Moreover, claims 1-3, 24, 26, 27,33-36,41-43 and 46-56, which depend variously from claim 44, are patentable for at least the same reasons.

8. Conclusion

In view of the foregoing, Applicant respectfully requests: the withdrawal of all objections and rejections of record; the allowance of all pending claims; and the holding of the application in condition for allowance.

Respectfully submitted on behalf of:
Agilent Technologies, Inc.

/s/William S. Francos/

by: William S. Francos (Reg. No. 38,456)

Date: September 16, 2008

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Appendix

Claims on Appeal

1. The method of claim 44, wherein said biological molecule is entrapped within pores of the gel, and the activity of the biological molecule is retained.
2. The method of claim 1, wherein after the sol-gel is formed and before the sol-gel is crushed, the sol-gel is aged for about two weeks.
3. The method of claim 1, wherein after the sol-the gel is formed and before the sol-gel is crushed, the sol-gel is aged at a temperature of from about 4° C to about 40 ° C.
9. A method for immobilizing a biological molecule in a porous inorganic matrix incorporated into a microanalytical device prepared according to the method of claim 44, said method for immobilizing the biological molecule comprising:
 - forming an aqueous composition comprising a tetraalkyl orthosilicate and a silane, wherein the silane is substituted with a C8-C24 alkyl group and substituted with at least two leaving groups selected from OR and halo, mixed with an acidified oxide salt solution;
 - adding to said composition an amount of the biological material in a physiologically acceptable-buffered solution wherein the resulting aqueous composition has a pH ranging from about 6 to about 8.5, said aqueous composition becoming turbid on being transformed into a polymerizing hydroxide solution and transforming to a gel;
 - shaping the gel produced into a final form; and
 - aging the gel;
 - wherein said biological molecule is entrapped within pores of the gel, and the activity of the biological molecule is retained; and wherein the porous inorganic matrix is formed in situ.
15. The method of claim 9, wherein the tetraalkyl orthosilicate is selected from the group consisting of tetra-ethyl orthosilicate, tetra-methyl orthosilicate, and combinations thereof.

16. The method of claim 1, wherein the sol is comprised of colloidal silica sol and a dissolved metal silicate.
17. The method of claim 16, wherein the metal silicate is sodium silicate.
18. The method of claim 1, wherein the sol comprises a tetraalkyl orthosilicate and a silane substituted with at least two leaving groups selected from the group consisting of OR and halo.
19. The method of claim 18, wherein the silane is substituted with a C₈-C₂₄ alkyl group.
20. The method of claim 19, wherein the alkyl group is C₁₈.
21. The method of claim 18, wherein the tetraalkyl orthosilicate is selected from the group consisting of tetra-ethyl orthosilicate, tetra-methyl orthosilicate, and combinations thereof.
24. The method of claim 1, wherein the particle size of the sol-gel is selected to produce pores when the sol-gel is aged, said pores being of a diameter which is approximately the same as the diameter of the biological molecule to be entrapped.
26. The method of claim 24, wherein the pores have an average diameter ranging from about 1 nm to about 100 nm.
27. The method of claim 1, wherein the pores have an average diameter ranging from about 2 nm to about 50 nm.
28. The method of claim 9, wherein the pores have a diameter which is approximately the same as the diameter of the biological molecule to be entrapped.

29. The method of claim 28, wherein the diameter of the pores is less than the diameter of the entrapped biomolecule.
30. The method of claim 9, wherein the gel produced prior to crushing is shaped into forms selected from the group consisting of a monolithic gel, thin film, or fiber.
31. The method of claim 9, wherein the pores have an average diameter ranging from about 1 nm to about 100 nm.
32. The method of claim 31, wherein the pores have an average diameter ranging from about 2 nm to about 50 nm.
33. The method of claim 24, wherein molecules having a mass of 3,000 Da or less can diffuse through the pores.
34. The method of claim 24, wherein molecules having a mass of 5,000 Da or less can diffuse through the pores.
35. The method of claim 24, wherein molecules having a mass of 10,000 Da or less can diffuse through the pores.
36. The method of claim 24, wherein molecules having a mass of 15,000 Da or less can diffuse through the pores.
37. The method of claim 28, wherein molecules having a mass of 3,000 Da or less can diffuse through the pores.
38. The method of claim 28, wherein molecules having a mass of 5,000 Da or less can diffuse through the pores.

39. The method of claim 28, wherein molecules having a mass of 10,000 Da or less can diffuse through the pores.

40. The method of claim 28, wherein molecules having a mass of 15,000 Da or less can diffuse through the pores.

41. The method of claim 1, wherein the biological molecule is selected from the group consisting of polynucleotides, enzymes, antibodies, coagulation modulators, cytokines, endorphins, peptidyl hormones, kinins, receptors, genes, gene fragments, cell fragments, membrane fragments, and solubilized membrane proteins.

42. The method of claim 41, wherein the enzyme is selected from the group consisting of RNase, DNase, telomerase, ligase, nuclease, ribonuclease; hydrogenase, dehydrogenase, aldase, amidase, aminotransferase, amylase, anhydrase, apyrase, arginase, aspartase, asparaginase, carboxylase, carboxypeptidase, catalase, cellulase, cholinesterase, acetylcholinesterase, deaminase, dextranase, dismutase, elastase, esterase, fumarase, glucosidase, hexokinase, isomerase, invertase, kinase, lactase, lipase, lysozyme, malase, naringinase, oxidase, oxygenase, papain, pectinase, peptidase, pepsin, peroxidase, phosphodiesterase, phosphotase, protease, reductase, transferase, tyrosinase, urase, trypsin, chymotrypsin, hydrolases, isomerases, proteases, ligases and oxidoreductases such as esterases, phosphatases, glycosidases and peptidases, superoxide dismutase, tissue plasminogen activator, renin, adenosine deaminase, alpha-glucocerebrosidase, asparaginase, dornase-alpha, hyaluronidase, elastase, trypsin, thymidine kinase, tryptophan hydroxylase, urokinase, kallikrein, bromelain, cathepsins B, D, G, C, clostripain, endoproteinase Arg C, endoproteinase Asp N, endoproteinase Glu C, endoproteinase Lys C, Factor Xa, proteinase K, subtilisin, thermolysin, acylamino acid releasing enzyme, aminopeptidases, carboxypeptidases, and pyroglutamate aminopeptidase.

44. A method of preparing a microanalytical device, comprising forming a sol-gel comprising an entrapped biological molecule, crushing the sol-gel to particulates having a diameter of from about 10 μm to about 80 μm , and forming the sol-gel particulates into a bed within the microanalytical device or on the surface of the microanalytical device.
45. The method of claim 44, wherein the form of said sol-gel is selected from the group consisting of a monolithic gel, thin film, and fiber.
46. A method of using a microanalytical device prepared according to the method of claim 44, comprising applying an analyte sample to the bed, optionally applying additional buffer solution to the bed, and analyzing the eluant from the bed.
47. The method of claim 44 or 46, wherein the bed on the microanalytical device is in the form of a microcolumn or microchannel.
48. The method of claim 44 or 46, wherein the bed on the microanalytical device is in the form of a microarray.
49. The method of claim 46, wherein the eluant is analyzed using mass spectrometry.
50. The method of claim 46, wherein the eluant is analyzed using micro or capillary electrophoresis.
51. The method of claim 46, wherein the interaction of any component in the sample with the entrapped biological molecule in the sol-gel is measured using a method selected from the group consisting of UV/Visible, Near IR, fluorescence, refractive index (RI) and Raman spectroscopies.
52. The method of claim 46, further comprising washing the sol-gel with a solution to elute analytes from the sol-gel, and analyzing the analytes.

53. The method of claim 52, wherein the analytes are analyzed using mass spectrometry.

54. The method of claim 52, wherein the analytes are analyzed using a method selected from the group consisting of UV/Visible, Near IR, fluorescence, refractive index (RI) and Raman spectroscopies.

55. The method of claim 44, wherein the microanalytical device is fabricated by a method selected from the group consisting of silicon micromachining, microlithography, molding and etching.

56. The method of claim 45, wherein the sol-gel is formed in situ on the microanalytical device.

58. In a microanalytical device comprised of a substrate and at least one feature selected from microchannels, microcolumns, and combinations thereof, the improvement which comprises incorporating into said at least one feature and/or onto a surface of the substrate a sol-gel having a biological molecule entrapped therein, wherein the sol-gel has been crushed into particulates having a diameter of from about 10 μm to about 80 μm and the particulates have been formed into a bed within the microanalytical device or on the surface of the microanalytical device.

59. The microanalytical device of claim 58, adapted for performing high throughput screening of samples.

Appendix

Evidence (None)

Appendix

Related Proceedings (None)